

# C-4 AND C-3 CARBOXYLATION CHARACTERISTICS IN THE GENUS *ZYGOPHYLLUM* (ZYGOPHYLLACEAE)<sup>1</sup>

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## ABSTRACT

Higher plants can be placed into two separate groups according to their pathways of photosynthetic CO<sub>2</sub> fixation. One group initially fix CO<sub>2</sub> into the 3-carbon acid 3-phosphoglycerate and are thus called C-3 plants. The others first fix CO<sub>2</sub> into one of three 4-carbon acids: aspartate, malate, and oxaloacetate and are therefore called C-4 plants. Several anatomical and physiological characteristics are correlated with the respective pathways. It has been suggested that the occurrence of these two different groups of plants may be taxonomically useful as each type is restricted to distinct plant taxa. Both types have been reported in at least four genera, but in each of these cases, species association is uncertain and taxonomic revision has been recommended. We found both C-4 and C-3 species in the genus *Zygophyllum* (Zygophyllaceae). This suggests that portions of the genus may have been misclassified. Our results also support the concept that some easily measured anatomical and physiological traits associated with the C-4 system can be used to clarify relationships in certain families. Use of these traits may be particularly useful in removing some uncertainties in classification of the Zygophyllaceae.

Plant species that form C-4 dicarboxylic acids as first products of photosynthesis (C-4 plants) have in common several anatomical and physiological characteristics that differ from plants with Calvin cycle photosynthesis (C-3 plants). Among these are carbon dioxide compensation points near 0  $\mu$ l CO<sub>2</sub>/liter of air and prominent chlorenchymatous vascular bundle sheaths. Downton and Tregunna (1968) suggested that C-4 traits could be taxonomically useful, as they are easily and experimentally discernible and they occur only in distinct taxa. Although a few genera do contain both C-4 and C-3 species, this has been reported only where species association is not certain and where revision has been recommended—*Panicum*, *Cyperus*, *Euphorbia*, and *Atriplex* (Downton *et al.*, 1969; Moss *et al.*, 1969; Tregunna & Downton, 1967).

We report here another genus which contains both C-4 and C-3 species, *Zygophyllum* (Zygophyllaceae). Our determination is based on leaf anatomy studies and CO<sub>2</sub> compensation point measurements.

## MATERIALS AND METHODS

For this report we studied seven species of the genus *Zygophyllum*: *Z. aurantiacum* F. Muell, *Z. coccineum* L., *Z. dumosum* Boiss, *Z. fabago* L., *Z. howittii* F. Muell, *Z. iodocarpum* F. Muell, and *Z. simplex* L. All plants were

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grown from seed sown in a 2:1 mixture of loam and peatmoss in 5-inch clay pots. These were placed in growth chambers with either 21-16° or 27-21°C day-night temperatures, a 16-hour daylength and a light intensity of 0.1 cal. cm<sup>-2</sup> min<sup>-1</sup> (400-700 nm). We made CO<sub>2</sub> compensation measurements with branches of plants that were approximately 40 days old. This consisted of measuring the equilibrium CO<sub>2</sub> concentration in a closed system containing an illuminated branch. The system consisted of a large stoppered glass test tube (leaf chamber) with two copper tubes extending through its rubber stopper. The chamber was immersed vertically in a water bath maintained at 29°C. Excised branches were placed in the leaf chamber with their stems resting in about 3 ml of water and were illuminated by two 150-watt incandescent lamps through a 10 cm water filter (light intensity of 2,400 ft-c). The copper tubes connected the leaf chamber to a pump which circulated the air in the system through an infrared gas analyser (Beckman model 215) and back to the chamber. The output from the analyser was recorded with a Sargent model SR recording potentiometer.

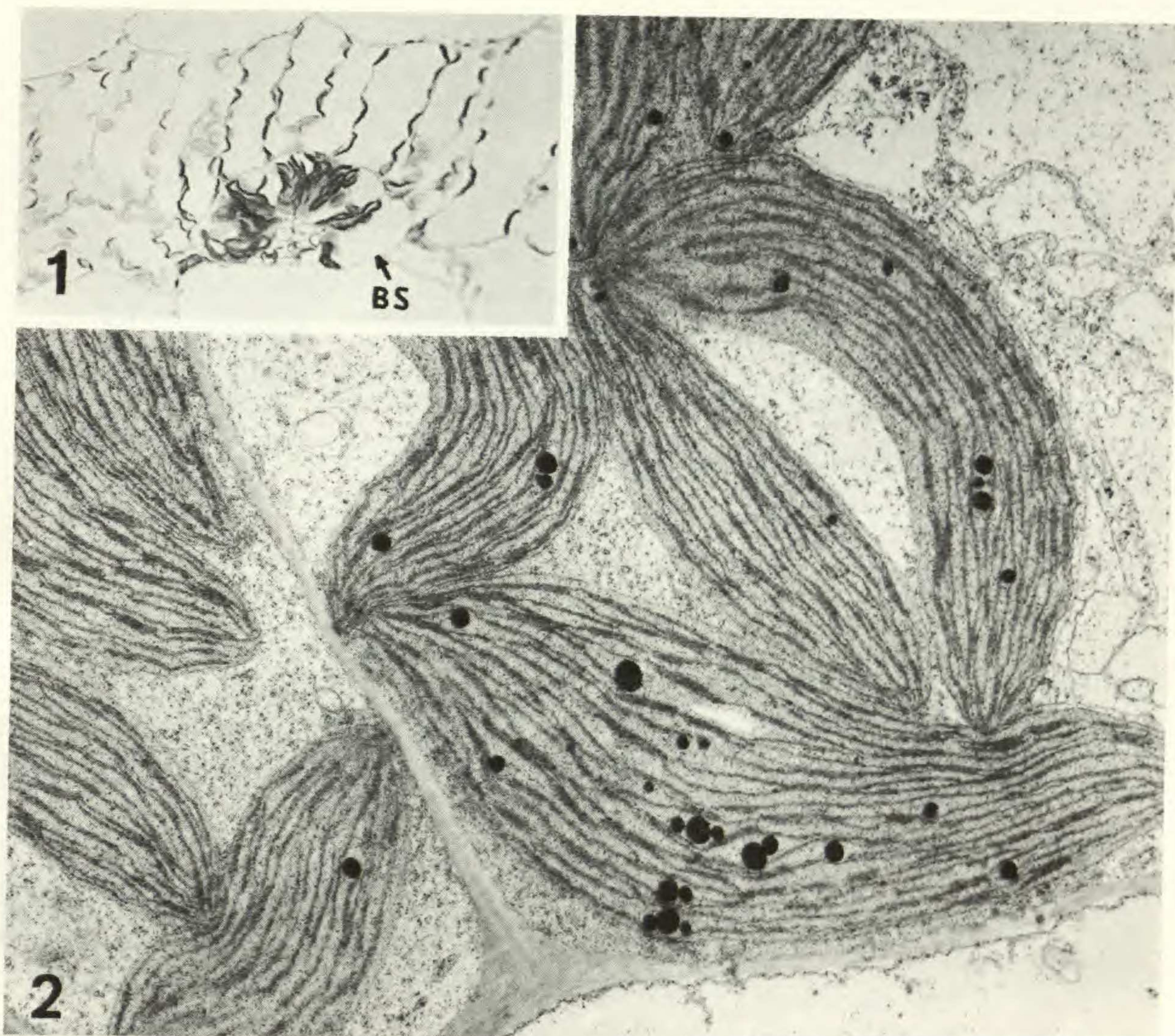
Freshly cut cross-sections of leaves of all species were examined with a light microscope for the presence or absence of C-4 type leaf anatomy. This included staining the sections with I<sub>2</sub>-KI so that starch-storage patterns could be observed. I<sub>2</sub>-KI solution was placed near one edge of the cover slip and drawn underneath by placing filter paper at the opposite side.

In addition, leaves of *Zygophyllum simplex*, which had a low CO<sub>2</sub> compensation point, were examined with the electron microscope. Leaf tissue was cut into segments approximately 1 mm square and fixed for two hours in a 2.5% glutaraldehyde solution freshly treated with BaCO<sub>3</sub> and buffered with 0.05 M NaPO<sub>4</sub> buffer (pH 6.8). It was then washed in buffer, post-fixed in OsO<sub>4</sub> (1% aqueous solution) for one hour, taken through a water-acetone dehydration series ending in dry acetone, and embedded in Spurr's embedding mixture (Spurr, 1969). Silver sections (approximately 800 Å thick) were cut using a glass knife on a Sorvall MT-2 ultramicrotome. These were mounted on uncoated # 400-mesh grids, post-stained in uranyl acetate (ten minutes) and lead citrate (five minutes), and viewed with a Hitachi HU-11-C electron microscope operated at 75 kV. Approximately 2 $\mu$ -thick sections of this same material were also cut and mounted in immersion oil on glass slides to obtain photomicrographs.

## RESULTS AND DISCUSSION

Of the seven *Zygophyllum* species which we studied, one, *Zygophyllum simplex*, had a low CO<sub>2</sub> compensation point (less than 5  $\mu$ l CO<sub>2</sub>/liter of air). It had leaves with prominent thick-walled vascular bundle sheaths. The cells of the bundle sheaths contained large chloroplasts (Fig. 1) which stained intensely with I<sub>2</sub>-KI indicating abundant starch. These associated traits suggest that *Zygophyllum simplex* is a C-4 species.

Electron microscopy showed that *Zygophyllum simplex* also had ultrastructural characteristics of C-4 plants. The bundle sheath cells contained numerous large chloroplasts which exhibited little grana formation (Fig. 2).



FIGURES 1-2.—1. Cross section of a leaf of *Zygothallum simplex*. The area shown is from the outer portion of the cylindrical leaf. The cells of the bundle sheath (BS) contain large chloroplasts. The bundle sheath is located at the inner edge of a layer of palisade cells. Epidermal cells are outside (above in figure), and large non-green parenchyma cells are inside (below in figure) this palisade layer.  $\times 88$ .—2. Electron micrograph of chloroplasts in the vascular bundle sheath cells of a *Zygothallum simplex* leaf. The chloroplasts are relatively starch free as the leaf was fixed in the early morning.  $\times 9,360$ .

These chloroplasts lacked an extensive peripheral reticulum, which is different from many C-4 species, although traces of a reticulum could be detected in some chloroplasts. These bundle sheath cells also contained numerous mitochondria grouped in large clusters near the cell walls (Fig. 3). Such groups of mitochondria were not found in central portions of the cells where there was a more uniform distribution of single mitochondria, or very small groups. All mitochondria had well-developed tubular cristae.

The species *Zygothallum aurantiacum*, *Z. coccineum*, *Z. dumosum*, *Z. fabago*, *Z. howittii*, and *Z. iodocarpum* had high  $\text{CO}_2$  compensation points (greater than  $40 \mu\text{l CO}_2/\text{liter of air}$ ). One of these high  $\text{CO}_2$  compensating species, *Z. coccineum*, had leaves similar to the low  $\text{CO}_2$  compensating species, *Z. simplex* (cylindrical, with chlorenchyma only around the outer edge), but without vascular bundle sheaths (Fig. 4). The other five species had oval or flattened

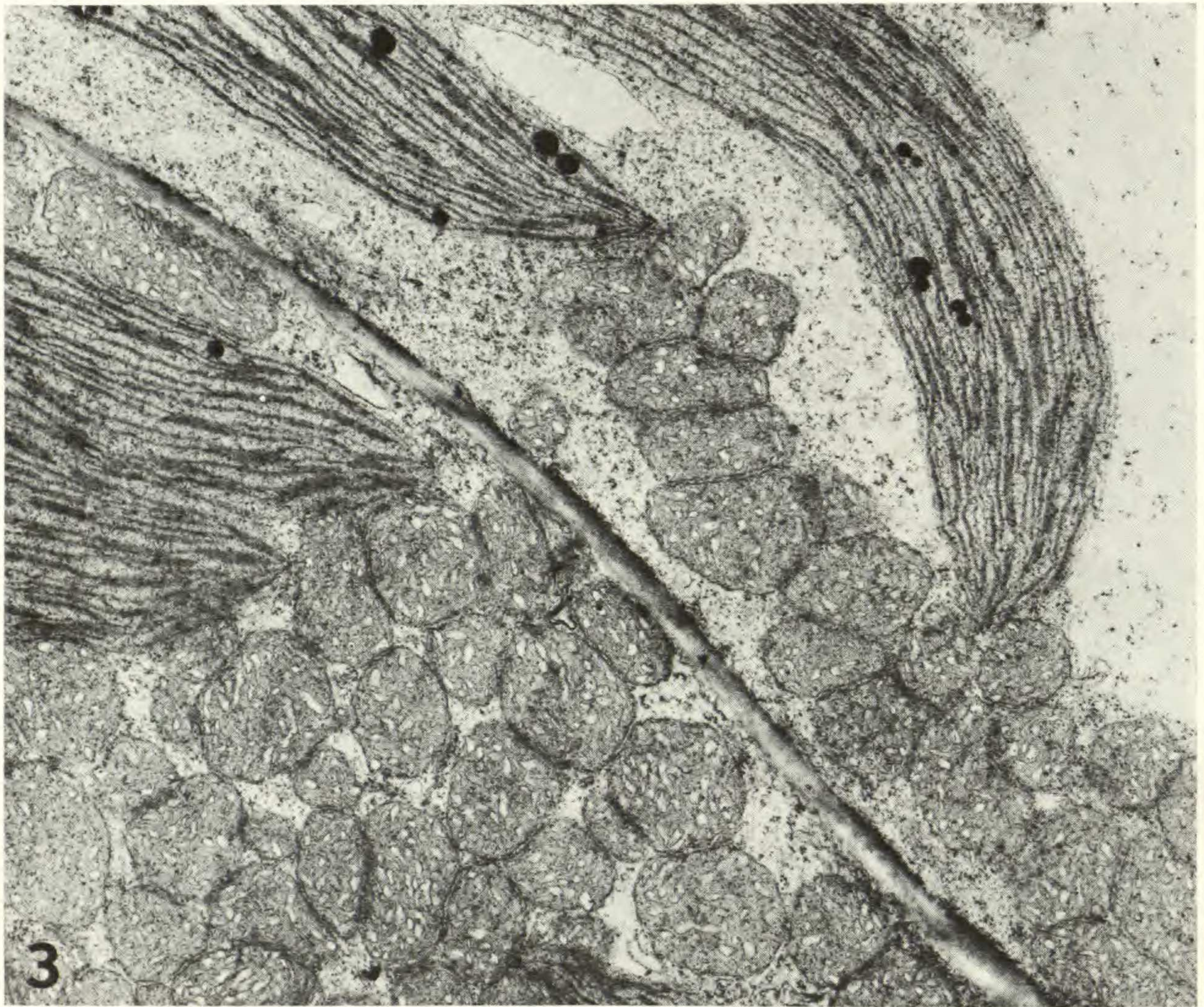


FIGURE 3. Electron micrograph showing the numerous and closely grouped mitochondria found within *Zygothallum simplex* bundle sheath cells. Such groups of mitochondria were found only next to cell walls.  $\times 6,320$ .

leaves composed of green palisade tissue and also lacking vascular bundle sheaths.  $I_2$ -KI staining indicated that there were no specialized areas of starch formation in leaves of any of the high  $CO_2$  compensating species. These six species were therefore determined to be C-3 type.

The fact that the genus *Zygothallum* contains both C-4 and C-3 species suggests that portions of it may have been misclassified. Porter (1963, 1969) has dealt with taxonomic problems of certain genera of the Zygothallaceae and has referred to several workers who recommend reclassification of much of the family. He states that cytological and genetical data are needed before one can knowingly discuss natural relationships. Since C-4 characteristics are cytologically prominent, we suggest that they could be used to assist in classification of *Zygothallum*.

In addition to *Zygothallum*, two other genera of the Zygothallaceae have also been found to contain C-4 traits. We reported earlier that *Tribulus terrestris* L. had a low  $CO_2$  compensation point and chlorenchymatous vascular bundle sheaths (Crookston & Moss, 1970). We have also observed these traits in *Tribulus cistoides* L., *T. hystrix* R. Br., *Kallstroemia maxima* L., *K. pubescens*

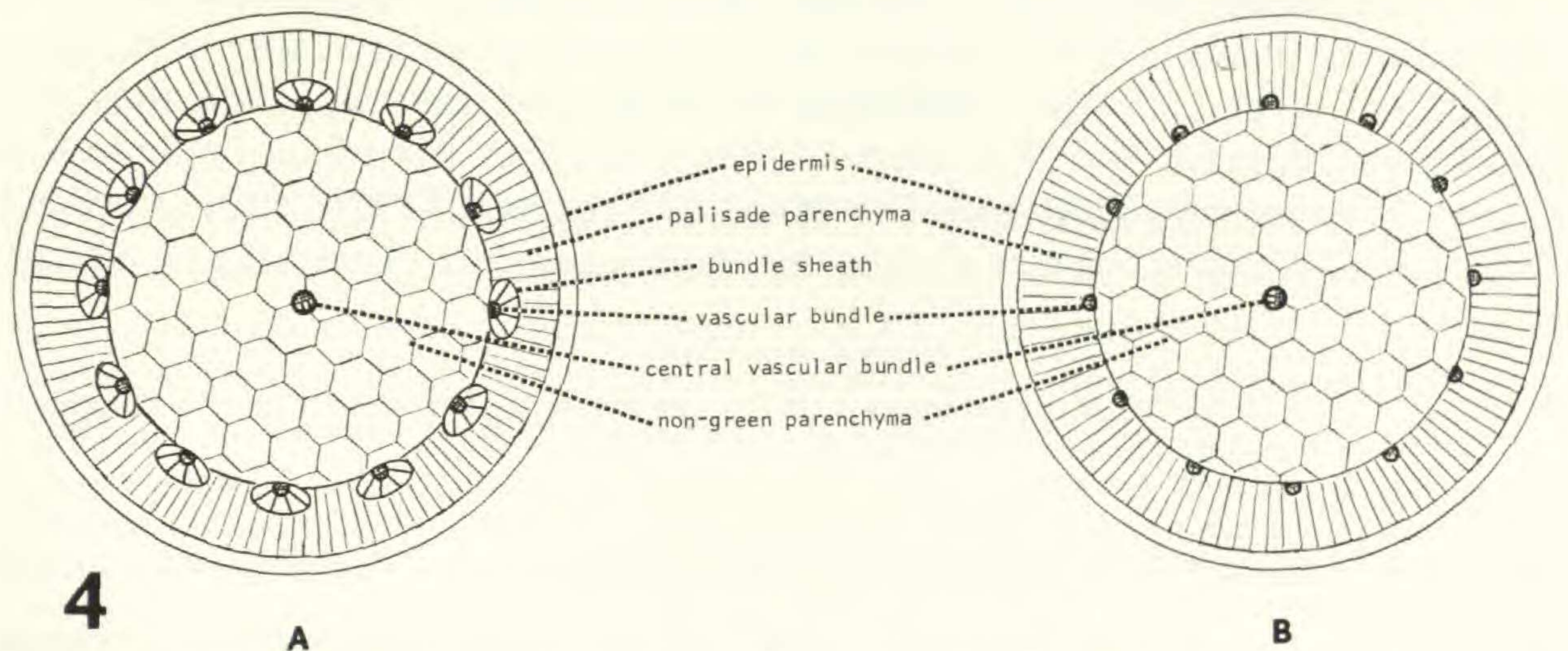


FIGURE 4. Diagrams of cross sections of two different *Zygothallum* leaves: *Z. simplex*, A; and *Z. coccineum*, B. Both of these leaves are cylindrical in shape. Note the presence of vascular bundle sheaths in *Z. simplex*.

(G. Don) Dandy, and *K. rosei* Rydb. In contrast to *Zygothallum* however, we have no indication that any C-3 species exist in *Tribulus* or *Kallstroemia*.

Porter (1969) suggests that *Tribulus* and *Kallstroemia*, along with *Kelleronia* and *Tribulopsis*, "constitute a natural group deserving recognition as a major subdivision of the family [Zygothallaceae]." Engler (1931) believed *Neoluederitzia* and *Sisyndite* also to be closely related to these four genera and said that they had all arisen from a "single primitive stock." Porter indicates that *Neoluederitzia* and *Sisyndite* have kinship elsewhere in the family, however, and do not belong in the group. Consideration of the presence or lack of C-4 traits by these genera should be helpful in confirming assumed relationships based, to present, on such characteristics as pollen type, leaf arrangement, and flower and fruit details.

An advantage of monitoring C-4 traits is that one can determine species type at almost any stage of growth and with certainty. There are numerous ways of making such determinations. According to Black and Mollenhauer (1971) the number and concentration of organelles in the bundle sheath, such as chloroplasts and mitochondria (Fig. 3-4), is the "most reliable anatomical criterion presently available for determining the photosynthetic capacity of a given plant" (C-4 or C-3 type). Electron microscopy is time consuming however, and we have found that when freshly cut cross-sections of leaves are viewed with a light microscope, C-4 sheaths appear very prominent and can be easily distinguished, even from the chlorenchymatous vascular bundle sheaths found in some C-3 species (Crookston & Moss, 1970). Since C-4 plants store starch primarily in their vascular bundle sheaths, staining cross sections of leaves with  $I_2$ -KI is often useful in confirming plant type determinations. Leaves of some C-4 species may be completely free of starch in early morning however, and they may contain considerable starch outside the sheath by late afternoon. Staining to detect starch storage patterns is therefore generally helpful, but it is not a completely reliable method of determination.

In addition to anatomical methods, we have found that measuring CO<sub>2</sub> compensation points is both a reliable and rapid method of determining whether a plant is C-4 or C-3 type. Individual measurements take approximately ten minutes and, thus far, low CO<sub>2</sub> compensation points have been found only in C-4 species. We therefore agree with Downton and Tregunna (1968) that examining plants for C-4 characteristics should, in many cases, be taxonomically useful.

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